Comparison of Agarose Gel with ABI PRISM® 5700

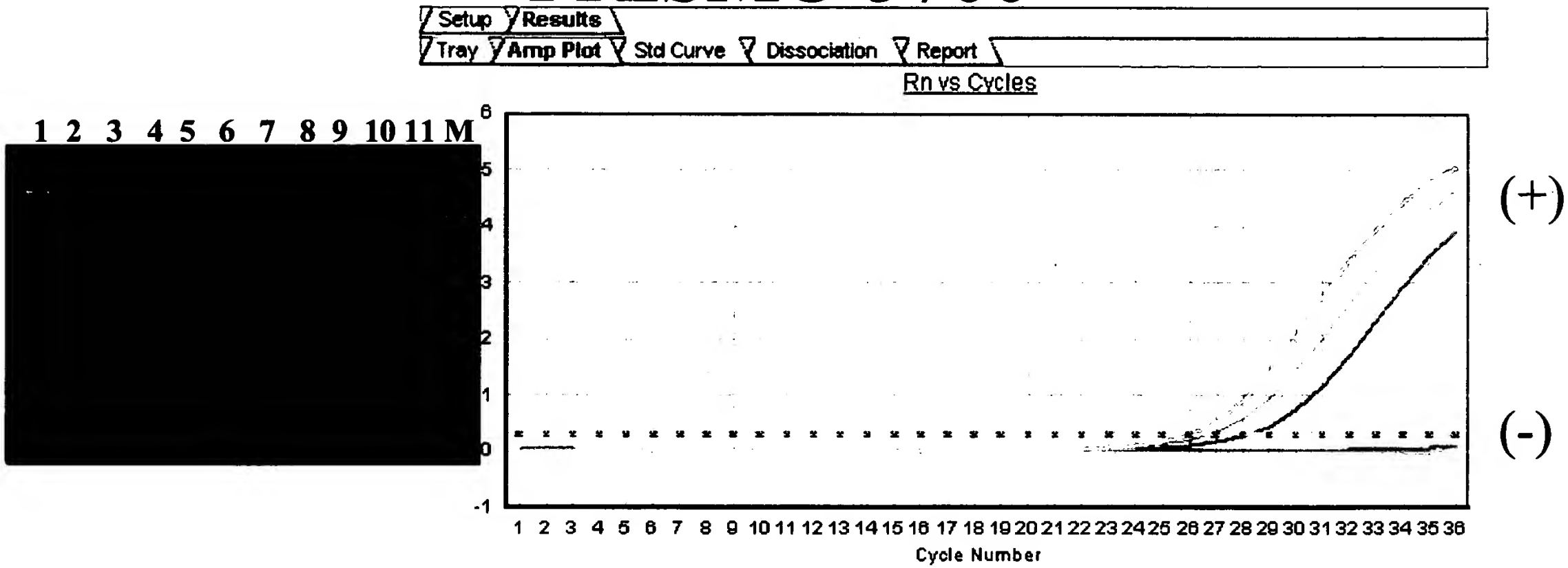

Figure 1. The agarose gel and the ABI PRISM® 5700 show different methods of evaluating PCR results. The gel shows the three positive PCR reactions (Lanes 1, 3 & 9), as well as a control ladder (Lane 12). The agarose gel also shows eight negative PCR reactions. The ABI PRISM® 5700 Sequence Detection System generates an Amplification Plot, which is a measurement of the increase in fluorescence of SYBR green. This increase correlates to an increase of PCR products. The above Amplification Plot shows the four positive reactions (+), and the eight negative reactions (-). The data in the Amplification Plot was collected during the PCR amplification, and the analyzed data was available immediately upon completion of the PCR reactions. The gel shows three positive reactions and not four because the positive control was not loaded, and the control ladder was run in its place.

Low Resolution Typing

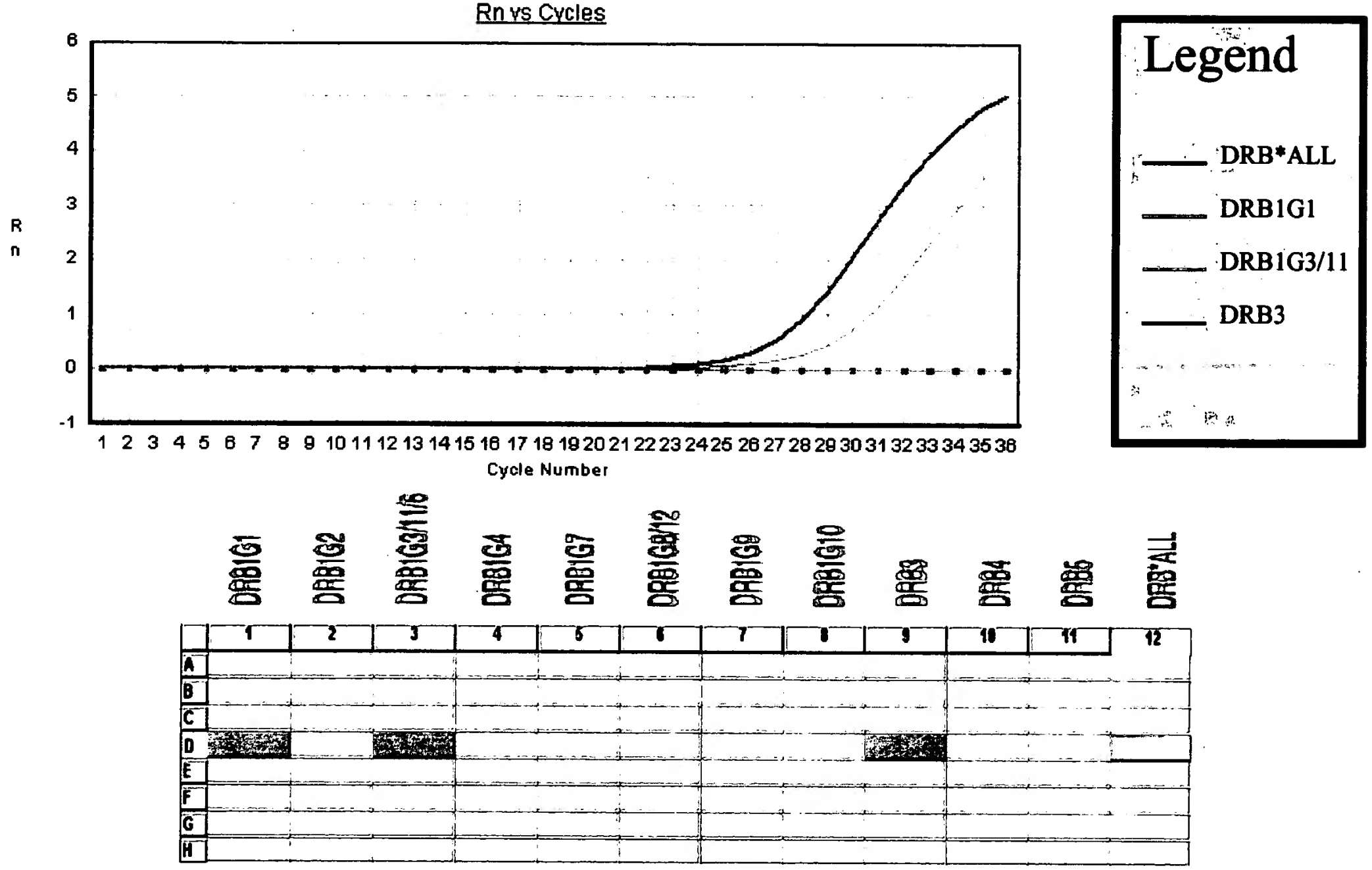

Figure 2. Based on the PCR results, this person is positive for DRB1G1, DRB1G3/11/6 and DRB3. This is an expected combination. This completes the low resolution typing of this individual. These same PCR products were then used for high resolution typing.

High Resolution Typing

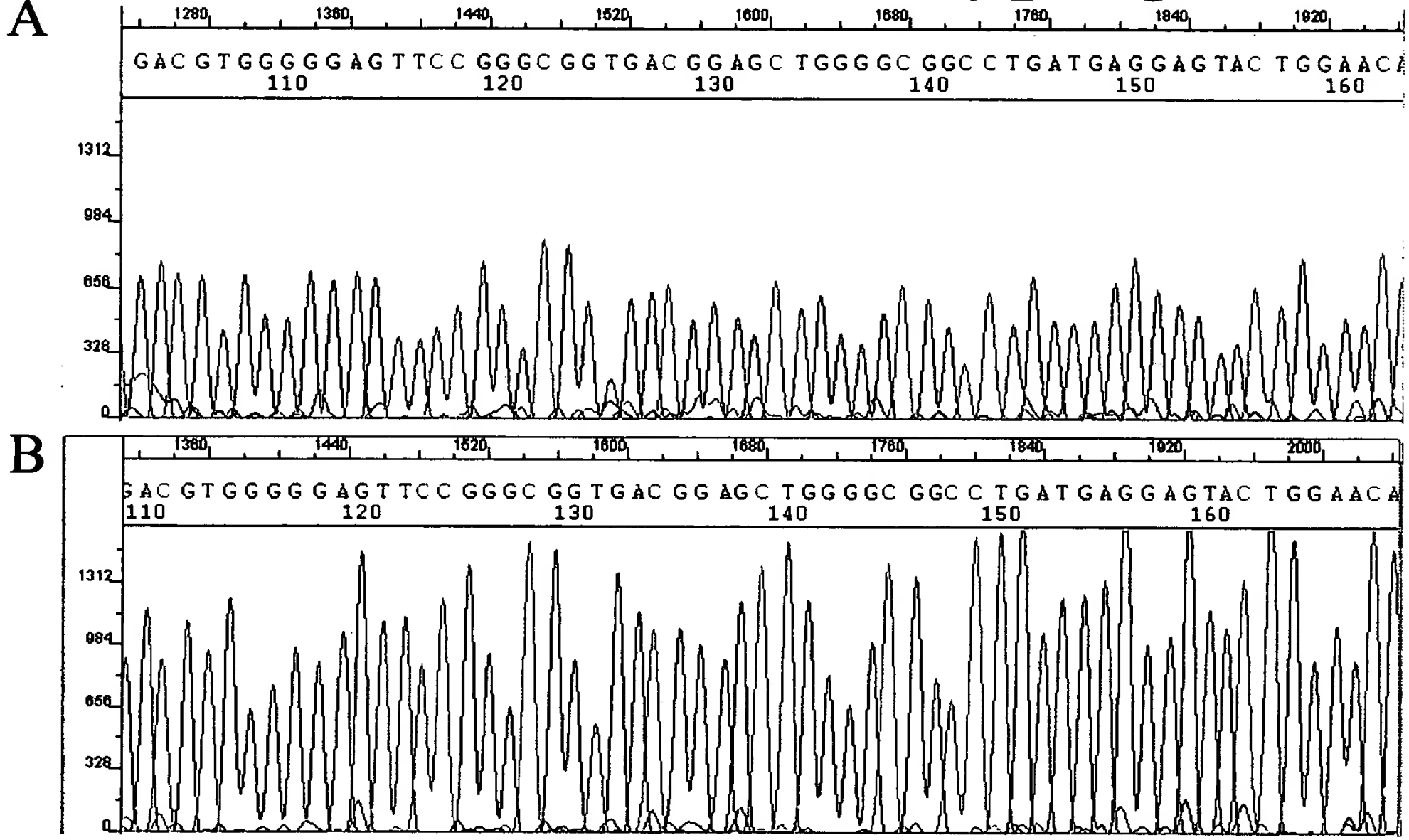

Figure 3. Panel A, shows sequence data from a PCR product produced using the standard HLA protocol. Panel B, shows sequence data from a PCR product generated using the modified SYBR/HLA protocol. Each sample was immediately sequenced after low resolution typing was completed. This comparison of data shows, the addition of SYBR® Green PCR Master Mix had no adverse effect on the sequencing reaction. This data was produced on the ABI PRISM® 3100 Genetic Analyzer.

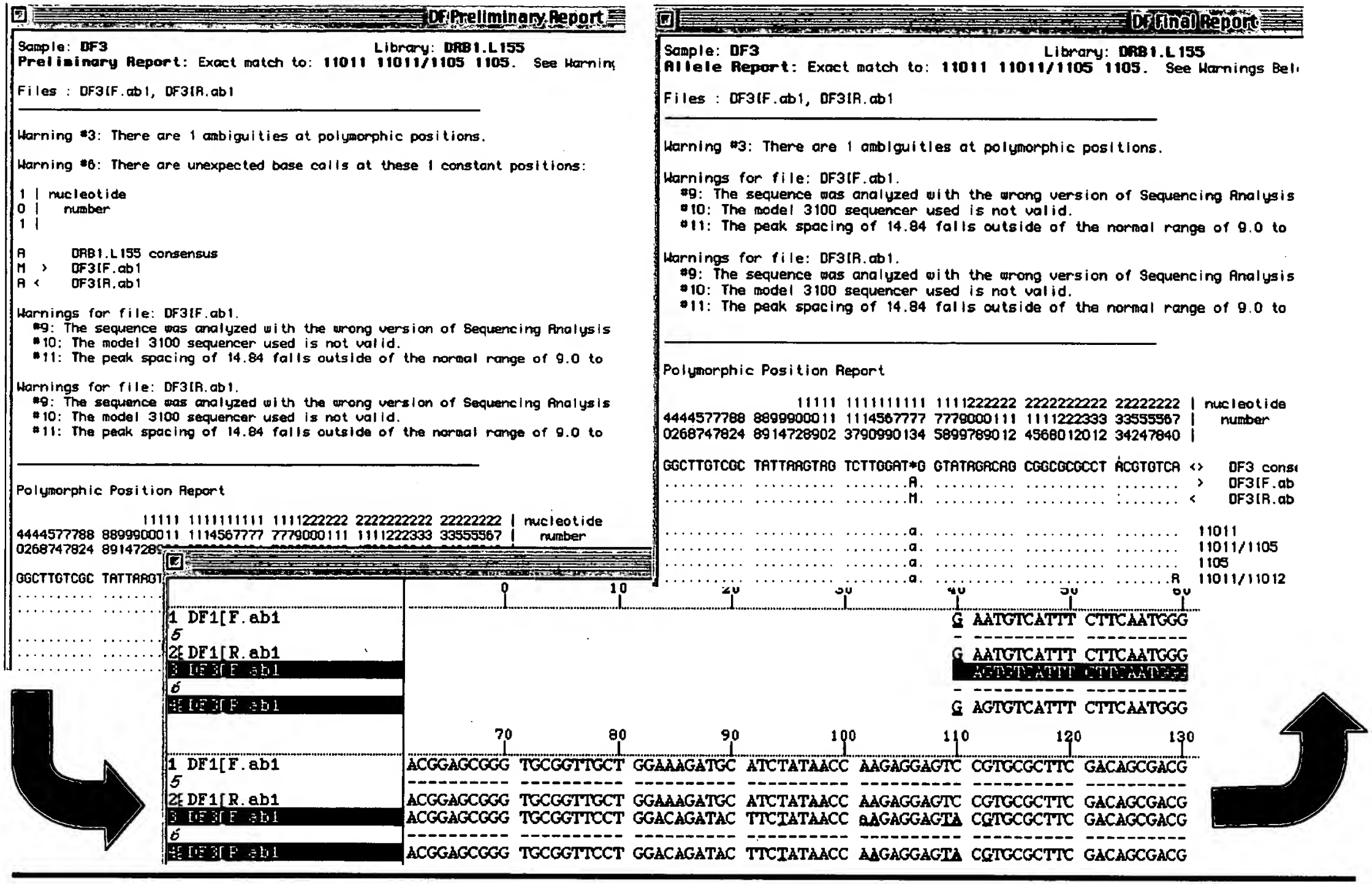

Figure 4. This panel shows the completion of the high resolution typing. The sequenced sample data was analyzed by the Applied Biosystems MatchTools™ software to get a Preliminary Report. The data was then edited in Applied Biosystems MT Navigator software, before being resubmitted to the Applied Biosystems MatchTools™ software for a Final Report. This sample was an exact match to 11011, 11011/1105, 1105.